EXHIBIT 7

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Consumption of Nitrate, Nitrite, and Nitrosodimethylamine and the Risk of Upper Aerodigestive Tract Cancer[1]

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Abstract

Evidence from animal studies indicates that various *N*-nitroso compounds are carcinogenic. We investigated whether consumption of foods and beverages containing nitrosodimethylamine, nitrites, and nitrates affected the risk of laryngeal, esophageal, and oral cancer. In a population-based case-control study in western Washington state, dietary consumption of these substances was measured in 645 cases (169 laryngeal, 125 esophageal, and 351 oral) and 458 controls. After adjustment for tobacco, alcohol, and other known risk factors, there was a 52% reduction in the risk of upper aerodigestive tract cancer for individuals who consumed higher amounts of nitrate (upper tertile) compared with the lowest tertile ($P < 0.001$ for trend). Nitrate intake was associated with a reduction in cancer risk at all three sites. The reduction in the risk of esophageal cancer with increasing nitrate consumption was more evident in frequent tea drinkers than in other subjects. There was no significant association between nitrite consumption and the risk of laryngeal or oral cancer. However, for individuals with a history of canker sores (an indicator of possible endogenous nitrosation), the risk of esophageal cancer was seven times greater in those with high *versus* low nitrite intake. Consumption of foods high in nitrosodimethylamine was associated with a 79% increased risk of upper aerodigestive tract cancer ($P = 0.037$ for trend). Cases consumed smoked fish more frequently than did controls [odds ratio (OR) = 3.03]. Daily intake of beer and of nitrite-containing meats were associated with an increased esophageal cancer risk (OR = 2.48 and 1.82, respectively). The odds ratio for daily beer intake was also elevated for individuals with cancer of the oral cavity (OR = 1.79). Subjects who consumed higher amounts of ascorbic acid from foods and supplements were less likely to develop upper aerodigestive tract cancer than were individuals with lower ascorbic acid intake ($P = 0.003$ for trend). These results indicate that nitrosation may be a factor in the etiology of upper aerodigestive tract cancers, especially esophageal cancer.

Introduction

Various *N*-nitroso compounds have been found to be carcinogenic to multiple organs in at least 40 animal species including higher primates (1). While some produce local tumors, others are systemic carcinogens (2). A common mechanism of action involves DNA alkylation, resulting in base modification (2). The cellular and molecular changes produced by some nitrosamines in animals have been found to be virtually identical to those in humans (3). In human ecological studies, a direct correlation has been found between exposure to either exogenous or endogenous nitrosamines and cancers of the stomach, esophagus, nasopharynx, urinary bladder, and liver (3). Despite the extensive information regarding the carcinogenicity of these compounds in animals, there have been few analytic studies investigating the risk to humans.

The typical diet in the United States consists of foods containing nitrates, nitrites, and *N*-nitroso compounds. Nitrates are found in many foods, with higher levels in vegetables such as beets, celery, rhubarb, turnip greens, radishes, and spinach (4). Nitrites are often added to retard microbial spoilage in processed meats and fish, although they are also found naturally in some grains and vegetables (4). Endogenous nitrite formation may occur by the reduction of nitrates by microflora in the salivary glands and in the stomach (5). Nitrites are known to combine with nitrosatable substances (amines and amides, generally present in protein-rich foods) to form *N*-nitroso compounds. Nitrosodimethylamine has been found in various processed meats, fish, and beer (6). In addition, endogenous nitrosation can be mediated by macrophages at inflamed or infected sites (6). Inhibition of *N*-nitrosation has been observed with ascorbic acid, tocopherols, retinoids, and phenols, as well as with tea (7).

It has been suggested that *N*-nitroso compounds are most effective as carcinogens in animals when taken p.o. and given in multiple small quantities over time (8). Since this is precisely what occurs over the course of a human lifetime, we conducted a study to evaluate the possibility of an increased risk of laryngeal, esophageal, and oral cancer through dietary consumption of nitrates, nitrites, and NDMA.[3] We chose to analyze NDMA in the present study

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[3] The abbreviations used are: NDMA, nitrosodimethylamine; CI, confidence interval; OR, odds ratio.

because it has been measured more extensively in foodstuffs than other *N*-nitroso compounds.

Materials and Methods

A population-based case-control study was conducted at the Fred Hutchinson Cancer Research Center in Seattle, Washington (9). Eligible subjects were residents of King, Pierce, and Snohomish counties in Washington state. All individuals newly diagnosed with laryngeal, esophageal, or oral cavity cancers (International Classification of Diseases for Oncology: 140.0–141.9; 143.0–146.9; 148.0–150.9; and 161.0–161.9) from September 1, 1983, through February 28, 1987, were identified using the Cancer Surveillance System, which is a part of the Surveillance, Epidemiology, and End Results Program. Eligible cases were individuals (*a*) with cancers of epithelial origin, (*b*) without a history of cancer at any site prior to the diagnosis of upper aerodigestive tract cancer, and (*c*) with a residential telephone. Individuals known to have AIDS were also excluded from the case group (approximately 12 individuals).

Controls were ascertained using random digit dialing of households in the same three-county area (10). The controls were frequency matched by age (5-year intervals) and by gender to the oral cancer cases. Similar to the case group, only individuals without a history of cancer at any site were included for this analysis.

Participants completed a food frequency questionnaire, indicating their usual eating habits 10 years prior to the interview. Foods known to contain nitrate, nitrite, or NDMA were specifically added to this questionnaire. Amounts of these substances were estimated using data from the report of the National Academy of Sciences (4) and surveys of NDMA content in meats and fish (11–14). NDMA concentrations in specific foods have been more widely published than other *N*-nitroso compounds and, therefore, were used in this study. Of the 125 food and beverage items on the questionnaire, 114 were estimated to contain nitrates, 72 were estimated to contain nitrites, and 18 were estimated to contain NDMA. With consideration of the usual serving size, the major sources of nitrates were dark green leafy vegetables, rhubarb, beets, celery, radishes, and broccoli. Primary sources of nitrites were processed meats and fish, with moderate amounts from beets, rhubarb, grains, and other vegetables. Amounts of NDMA were estimated from processed meats and fish and from beer. Beer is not known to be high in nitrate or nitrite, but it contained considerable amounts of NDMA during the time frame of this study (4). In addition, specific questions were asked regarding the consumption of smoked and dried meats, smoked fish, and pickled meats and fish.

Consumption of beverages, including drinking water, was also ascertained from the food frequency questionnaire. The amount of nitrate from these items was calculated by using the National Academy of Sciences (4) estimate of nitrate in United States drinking water (1.3 mg/liter). Since the source of drinking water in the study area is mountainous runoff, and contamination of reservoirs from nitrogen-containing fertilizers is not an appreciable concern, the United States estimate is probably an overestimate of the nitrate from water consumption. Most of the subjects lived within three cities, Seattle, Tacoma, and Everett. Although it is possible that some subjects had prior exposure to high nitrate-containing water earlier in life or had well water containing nitrate, the number of these subjects, if any, was probably small.

Ascorbic acid intake was estimated using information from food and supplement intake combined. Tea consumption was recorded for two categories, herbal and regular tea (black and/or green). Information regarding use of tobacco products, alcohol, occupation, medical history, dentition, and demographic information was also collected by personal interview. Tobacco use was measured using pack-years of cigarette use (continuous variable) and by using a dichotomous variable indicating ever-use of tobacco products (cigarettes, cigars, pipes, chewing tobacco, snuff, and minipouches) *versus* never-use. Alcohol use was quantified by using the number of drinks consumed/day (beer, wine, and hard liquor) multiplied by the years of use to yield drink-years of alcohol use. Level of education was indicated by a dichotomous variable, high school education or less *versus* college/technical school education or more. Body mass index [weight (kg)/height (m^2)] was included as a continuous variable which indicated the recollection of height and weight of the subject 1 year prior to the diagnosis for cases and 1 year prior to the interview for controls.

There were 960 eligible cases and 625 eligible controls identified. Physician or subject refusal occurred for 17% of the cases and 18% of the controls. Individuals with 5 or more unknown food items on the questionnaire were excluded, as well as those unable to complete a questionnaire due to language or medical problems. There were 645 cases (169 laryngeal, 125 esophageal, and 351 oral) and 458 controls who completed enough information from the dietary questionnaire to estimate nitrate, nitrite, and NDMA consumption (67% of the eligible cases and 73% of the eligible controls).

Analysis of the data proceeded in three phases: (*a*) a univariate analysis to determine the distributions of the variables; (*b*) bivariate analysis to determine interrelationships between variables and the presence of outliers; and (*c*) a multivariate analysis using unconditional logistic regression as described by Breslow and Day (15). OR and 95% CI were calculated for food groups known to contain nitrates, nitrites, and NDMA. Odds ratios were also calculated for estimates of total nitrate, nitrite, and NDMA consumption. The exposure variables were categorized into tertiles using the distribution of the control group. Since ascorbic acid and tea have both been found to inhibit nitrosation (7), effect modification by these two variables was investigated. In addition, inflammation may result in endogenous formation of *N*-nitroso compounds; therefore, interactions between a history of oral infection and consumption of *N*-nitroso precursors were examined. Tobacco is known to contain various *N*-nitroso compounds, and odds ratios were examined separately for tobacco users and nonusers as well. Since the etiology of esophageal adenocarcinomas may differ from squamous cell tumors of the esophagus, odds ratios were calculated for esophageal adenocarcinomas.

Final logistic models contained the variables nitrate, nitrite, NDMA, age, gender, educational level, tobacco use (pack-years), alcohol use (drink-years), body mass index (kg/m^2), and energy intake (kcal/day). Adjustment for other dietary factors (β-carotene, niacin, riboflavin, zinc, iron, and calcium) did not appreciably change the results.

Table 1 Odds ratios and 95% confidence intervals for consumption of food groups containing nitrates, nitrites, and nitrosodimethylamine and upper aerodigestive tract cancer

Type of food	Cases/controls higher consumption[a]	Cases/controls reference category[a]	OR[b]	95% CI
Nitrate-containing vegetables with higher ascorbic acid content[c]	320/300	226/128	0.71	0.51–0.97
Nitrate-containing vegetables with lower ascorbic acid content[c]	465/393	81/35	0.61	0.37–1.01
Smoked or dried meats	21/13	525/415	1.08	0.45–2.57
Smoked fish	13/7	533/421	3.03	1.04–8.87
Pickled meats	7/2	539/426	2.38	0.32–17.7
Pickled fish	3/1	642/457	2.13	0.17–112
Nitrite-containing meats[d]				
Larynx	35/89	116/339	0.86	0.49–1.50
Esophagus	34/89	63/339	1.82	0.99–3.34
Oral cavity	72/89	226/339	0.88	0.55–1.39
Beer				
Larynx	41/48	110/380	1.52	0.83–2.79
Esophagus	31/48	66/380	2.48	1.32–4.66
Oral cavity	90/48	208/380	1.79	1.11–2.88

[a] Higher consumption was 1 or more times/week for vegetables and for smoked or pickled foods; reference category was <1 time/week. Higher consumption was 1 or more times/day for nitrite-containing meats and beer; reference category was <1 time/day.
[b] All odds ratio were adjusted for age, gender, pack-years of cigarettes, drink-years of alcohol, energy intake, ascorbic acid intake, body mass index, and level of education.
[c] Nitrate-containing vegetables with higher ascorbic acid content were: broccoli; spinach; romaine lettuce; kale or collards; mustard, turnip, dandelion, chard or beet greens. Nitrate-containing vegetables with lower ascorbic acid content were: beets, celery, radishes, iceberg lettuce, and rhubarb.
[d] Nitrite-containing meats were: hot dogs, bacon, ham, corned beef, sausage, salami, bologna, cold cuts, meat spread, canned meats, liverwurst or braunschweiger, and pizza with cheese and meat.

Results

The cases (three sites combined) were similar to controls with respect to age, gender, and race (16). Esophageal cancer cases were slightly older than controls (69 *versus* 57% were ≥60 years of age, respectively). Laryngeal cancer cases contained a larger proportion of men (81%) than did the controls (67%). Cases were less educated than controls; 58% of the controls had at least some formal education beyond high school, while only 37% of the laryngeal cases, 31% of esophageal cases, and 40% of the oral cases had reported some higher education. Individuals in the case groups were more likely to use tobacco products and alcohol than were controls. While 42% of individuals without cancer had used cigarettes for 20 or more pack-years, 78% of the cases had such a history. Only 12% of the controls had ≥65 drink-years of total lifetime alcohol use, while 42% of the cases had a similar history. Cases also reported a lower body mass index (mean, 25.2 for larynx; 25.0 for esophagus; and 24.6 kg/m^2 for oral cavity) than did controls (mean, 26.1 kg/m^2).

Odds ratios for specific foods known to be high in nitrates, nitrites, and *N*-nitroso compounds are given in Table 1. Consumption of nitrate-containing vegetables was associated with a decreased risk of upper aerodigestive tract cancer (OR, 0.51 for all sites combined), which was evident for each cancer site (ORs not shown). This was irrespective of whether the vegetables contained a higher amount of ascorbic acid (OR, 0.71) or not (OR, 0.61). There was no statistically significant association between smoked or dried meats and cancer (Table 1). However, subjects who ate smoked fish at least once per week were three times as likely to develop upper aerodigestive tract cancer as individuals who ate smoked fish less than once per week. The number of individuals who consumed pickled foods on a weekly basis was small, and the odds ratios, although elevated, were not statistically significant. For esophageal cancer cases there appeared to be an elevation in risk for daily consumption of nitrite-containing meats (OR, 1.82) that was not evident for laryngeal (OR, 0.86) or oral (OR, 0.88) cancers.

Daily consumption of beer was associated with an elevated odds ratio for esophageal (OR, 2.48) and oral (OR, 1.79) cancers after adjustment for pack-years, drink-years of alcohol use, energy intake, ascorbic acid intake, body mass index, and level of education (Table 1). Although the odds ratio was also elevated for daily consumption of beer in laryngeal cancer cases (OR, 1.52), it was not statistically significant. When drink-years of alcohol use was excluded from the model, daily consumption of beer was significantly associated with each site (OR, 2.23 for larynyx; 4.58 for esophagus, and 2.96 for oral cavity).

Table 2 indicates that subjects who consumed higher amounts of nitrates were less likely to develop upper aerodigestive tract cancer than were individuals who consumed lower amounts. Those individuals in the highest tertile of consumption had less than one-half of the risk of upper aerodigestive tract cancer compared with subjects in the lowest tertile for each of the three sites studied. The odds ratios for all three sites combined were 1.0 (low-reference category), 0.67, and 0.48 ($P < 0.001$, test for trend).

There was no statistically significant association between nitrite consumption and cancer at these sites (Table 2). The odds ratios decreased with increasing nitrite level for laryngeal and oral cancer, whereas they increased with each nitrite level for esophageal cases.

There was a 79% increased risk of upper aerodigestive tract cancer (all sites combined) in individuals who consumed higher amounts of NDMA compared to individuals who consumed the lowest amounts of NDMA ($P = 0.037$ for trend). This association was quite consistent across each cancer site (Table 2). Effect modification by tobacco use was investigated, but no statistically significant interactions were observed for any of the three cancer sites.

Table 2 Odds ratios and 95% confidence intervals for daily consumption of nitrate, nitrite, and nitrosodimethylamine and cancers of the larynx, esophagus, and oral cavity

	No. of Controls	Larynx			Esophagus			Oral cavity		
		No. of Cases	OR[a]	95% CI	No. of Cases	OR[a]	95% CI	No. of Cases	OR[a]	95% CI
Nitrate										
<134 mg	144	67	1.00		33	1.00		113	1.00	
134–226 mg	144	49	0.50	0.29–0.88	39	0.71	0.38–1.33	109	0.66	0.43–1.01
>226 mg	140	35	0.42	0.22–0.80	25	0.44	0.24–0.93	76	0.46	0.28–0.76
			(trend: $P = 0.005$)			(trend: $P = 0.078$)			(trend: $P = 0.001$)	
Nitrite										
<1.06 mg	143	48	1.00		26	1.00		112	1.00	
1.06–1.60 mg	134	50	0.98	0.54–1.79	28	1.17	0.57–2.38	92	0.96	0.61–1.51
>1.60 mg	151	53	0.67	0.34–1.34	43	1.58	0.73–3.44	94	0.66	0.39–1.12
			(trend: $P = 0.107$)			(trend: $P = 0.200$)			(trend: $P = 0.099$)	
NDMA										
<0.06 μg	134	32	1.00		22	1.00		68	1.00	
0.06–0.179 μg	147	37	1.11	0.57–2.13	23	1.31	0.60–2.85	75	1.51	0.92–2.48
>0.179 μg	147	82	1.70	0.91–3.18	52	1.86	0.87–3.95	155	1.82	1.10–3.00
			(trend: $P = 0.258$)			(trend: $P = 0.063$)			(trend: $P = 0.118$)	

[a] Odds ratios were adjusted for age, gender, pack-years of cigarettes, drink-years of alcohol, energy intake, ascorbic acid intake, body mass index, and level of education.

Table 3 Odds ratios for cancers of the larynx, esophagus and oral cavity by daily consumption of nitrate, nitrite, nitrosodimethylamine, and ascorbic acid

	Larynx		Esophagus		Oral cavity	
	High ascorbic acid[a] OR[b]	Low ascorbic acid[a] OR[b]	High ascorbic acid[a] OR[b]	Low ascorbic acid[a] OR[b]	High ascorbic acid[a] OR[b]	Low ascorbic acid[a] OR[b]
Nitrate						
<134 mg	1.0 (referent)	0.87	1.0 (referent)	1.63	1.0 (referent)	1.71
134–226 mg	0.34[c]	0.59	0.73	1.44	0.89	1.27
>226 mg	0.35[c]	0.46	0.44	1.27	0.73	0.65
Nitrite						
<1.06 mg	1.0 (referent)	1.65	1.0 (referent)	2.93	1.0 (referent)	2.40[c]
1.06–1.60 mg	1.04	1.22	2.24	2.23	1.62	1.64
>1.60 mg	0.65	0.95	1.49	5.07[c]	1.01	1.30
NDMA						
<0.06 μg	1.0 (referent)	1.58	1.0 (referent)	1.43	1.0 (referent)	1.49
0.06–0.179 μg	1.03	1.57	0.64	3.10[c]	1.17	2.24[c]
>0.179 μg	1.58	2.02	1.61	2.96[c]	1.60	2.05[c]

[a] High ascorbic acid was >195 mg/day. Low ascorbic acid was <195 mg/day.
[b] Odds ratios were adjusted for age, gender, pack-years of cigarettes, drink-years of alcohol, energy intake, body mass index, and level of education.
[c] $P < 0.05$.

When analyses were restricted to the 33 persons (31 men and 2 women) with esophageal adenocarcinomas, the crude associations with nitrate, nitrite, and NDMA were similar to those for esophageal squamous cell carcinomas. However, the small numbers prohibited meaningful adjustment for other risk factors.

Ascorbic acid intake (food and supplements combined) was inversely associated with cancer risk. The odds ratios by tertiles of consumption were 1.00 (reference = low consumption), 0.70, and 0.55 ($P = 0.003$ for trend) for the three sites combined. The reduction in the odds ratios with increasing ascorbic acid intake was evident for each cancer site, although the test for trend was only statistically significant for esophageal cancer ($P < 0.001$ for trend) and laryngeal cancer ($P = 0.024$ for trend) but not oral cancer ($P = 0.103$ for trend). Modification of the associations with nitrates, nitrites, and NDMA by ascorbic acid intake was also investigated (Table 3). Although there were no statistically significant interactions at the 0.05 level, the data suggest that individuals with both low ascorbic acid intake and high NDMA intake may be at increased risk of upper aerodigestive tract cancers. There was also a strong association between esophageal cancer and diets low in ascorbic acid and high in nitrite (OR, 5.07). Subjects with high intake of both nitrate and ascorbic acid tended to have the lowest risk of cancer, which was consistent across cancer sites.

More frequent tea drinkers were less likely to develop aerodigestive tract cancer than individuals who drank tea less than once per week (crude OR, 0.67; 95% CI, 0.52–0.87 for regular tea and crude OR, 0.55; 95% CI, 0.33–0.92 for herbal tea). However, when adjusted for the other covariates (see footnote in Table 2), the odds ratios were not statistically significant (OR, 0.79; 95% CI, 0.56–1.11 for regular tea and OR, 1.60; 95% CI, 0.84–3.04 for herbal tea). Tea drinking did not significantly modify the observed associations of nitrate, nitrite, or

Table 4 Odds ratios and 95% confidence intervals for esophageal cancer and daily consumption of nitrate, nitrite and nitrosodimethylamine by tea intake[a]

	Level	Subjects who drank tea < once/week			Subjects who drank tea 1 or more times/week		
		No. of Cases/controls	OR[b]	95% CI	No. of Cases/controls	OR[b]	95% CI
Nitrate	<134 mg	21/100	1.00		12/44	1.00	
	134–226 mg	32/96	0.95	0.45–2.00	7/48	0.32	0.10–1.04
	>226 mg	20/83	0.69	0.30–1.61	5/57	0.13	0.03–0.52
			(trend: $P = 0.976$)			(trend: $P = 0.001$)	
Nitrite	<1.06 mg	19/105	1.00		7/38	1.00	
	1.06–1.60 mg	20/80	1.49	0.64–3.47	8/54	0.66	0.19–2.30
	>1.60 mg	34/94	2.12	0.88–5.11	9/57	0.74	0.21–2.59
			(trend: $P = 0.046$)			(trend: $P = 0.306$)	
NDMA	<0.06 μg	16/93	1.00		6/41	1.00	
	0.06–0.179 μg	19/88	1.92	0.77–4.81	4/59	0.52	0.11–2.33
	>0.179 μg	38/98	1.93	0.81–4.58	14/49	1.81	0.50–6.46
			(trend: $P = 0.093$)			(trend: $P = 0.615$)	

[a] Data were for subjects who drank regular (nonherbal) tea.
[b] Odds ratios were adjusted for age, gender, pack-years of cigarettes, drink-years of alcohol, energy intake, ascorbic acid intake, body mass index, and level of education.

Table 5 Odds ratios and 95% confidence intervals for esophageal cancer and daily consumption of nitrate, nitrite and nitrosodimethylamine by a history of canker sores[a]

	Level	Subjects with a history of canker sores			Subjects without a history of canker sores		
		No. of Cases/controls	OR[b]	95% CI	No. of Cases/controls	OR[b]	95% CI
Nitrate	<134 mg	4/80	1.00		29/61	1.00	
	134–226 mg	6/65	1.18	0.28–4.95	33/76	0.56	0.27–1.15
	>226 mg	11/82	1.42	0.38–5.24	13/58	0.26	0.10–0.69
			(trend: $P = 0.257$)			(trend: $P = 0.007$)	
Nitrite	<1.06 mg	2/78	1.00		24/64	1.00	
	1.06–1.60 mg	7/75	5.49	0.97–30.96	20/56	0.77	0.33–1.81
	>1.60 mg	12/74	7.33	1.30–41.43	31/75	1.08	0.46–2.54
			(trend: $P = 0.020$)			(trend: $P = 0.933$)	
NDMA	<0.06 μg	4/79	1.00		17/54	1.00	
	0.06–0.179 μg	5/75	1.84	0.42–8.04	18/69	1.12	0.45–2.82
	>0.179 μg	12/73	2.85	0.73–11.12	40/72	1.58	0.66–3.74
			(trend: $P = 0.113$)			(trend: $P = 0.178$)	

[a] Subjects who reported ever having canker sores inside the mouth.
[b] Odds ratios were adjusted for age, gender, pack-years of cigarettes, drink-years of alcohol, energy intake, ascorbic acid intake, body mass index, and level of education.

NDMA with oral or laryngeal cancer. However, for esophageal cancer (Table 4) there was evidence that more frequent tea drinkers had a greater reduction in the risk of esophageal cancer with increasing nitrate consumption than did other subjects ($P = 0.024$ for interaction). The odds ratios for nitrite were also lower for frequent tea drinkers than other subjects, although these differences could be due to chance.

Since nitrosation may occur at infected or inflamed sites, we investigated whether the presence of cold sores or canker sores inside the mouth affected the risk estimates for nitrate, nitrite, and NDMA consumption. There was no statistically significant effect modification by cold sore status (ever-had *versus* never-had) for the risk estimates for laryngeal, esophageal, and oral cancer. However, a history of cold sores was inversely related to esophageal cancer risk (OR, 0.56; 95% CI, 0.33–0.96). Furthermore, a history of canker sores (ever-had *versus* never-had) was inversely related to esophageal (OR, 0.33) and oral (OR, 0.65) cancers. There was no statistically significant effect modification by a history of canker sores for subjects with laryngeal and oral cancers. However, as shown in Table 5, the risk of esophageal cancer appeared to decrease with higher nitrate consumption in subjects who never had canker sores (OR, 0.26), but increased somewhat in those with a history of canker sores ($P = 0.030$ for interaction). Similar odds ratios for high nitrate consumption in individuals who never had canker sores were evident for laryngeal (OR, 0.30; 95% CI, 0.13–0.69) and oral (OR, 0.36; 95% CI, 0.18–0.69) cancers, although there was no significant interaction by canker sore status.

The risk of esophageal cancer increased with higher nitrite consumption in subjects with a history of canker sores (OR, 7.33; Table 5) but not in subjects who never had canker sores ($P = 0.039$ for interaction). A similar, although nonsignificant, pattern was seen for laryngeal cases but not for individuals with oral cancer.

Discussion

We found that consumption of vegetables high in nitrates was associated with a reduction in the risk of upper aerodigestive tract cancer, and that consumption of foods high in NDMA resulted in an elevated risk of cancer at these

sites. Intake of vegetables, in general, has been found to reduce the risk of gastrointestinal and respiratory tract cancers in a number of other studies (17, 18). We initially hypothesized that nitrate intake would be associated with higher risk since nitrates may be converted to nitrites which, in turn, may combine with substrates to form *N*-nitroso compounds. This was not confirmed in this study. The nitrate-containing vegetables may contain other substances which may reduce cancer risk or, alternatively, individuals who consume these vegetables may differ from other subjects by factors not measured in this study. The results in Table 1 indicate that the protective agent(s) in vegetables may not (solely) be ascorbic acid, since the protective effect was also seen in vegetables low in ascorbic acid. Researchers have indicated that there may be other nonnutritive chemopreventive agents in vegetables (19). Adjustment was made for possible confounders such as tobacco use; alcohol use; intake of β-carotene, niacin, riboflavin, zinc, iron, calcium; and total kcal. These factors did not account for the association observed. Two studies reported a decreased risk of gastric cancer for higher nitrate consumption (20, 21), while other studies have not (22–24). Jain *et al.* (25) reported a possible protective effect of eating nitrate-containing vegetables ($P = 0.0006$ for trend) for lung cancer, which was more evident in adenocarcinomas than in squamous cell tumors.

Consumption of foods containing NDMA was associated with a higher risk of developing upper aerodigestive tract cancer in this study. The odds ratios for consumers of some nitrosamine- and nitrite-containing foods (Table 1) were elevated, although significantly so only for those who ate smoked fish. Pickled meats and fish were infrequently eaten in this population so the confidence intervals were wide. Daily consumption of nitrite-containing meats may be associated with an increased risk of esophageal cancer, although the odds ratio was of borderline significance. These results are broadly consistent with the results of other studies. Zheng *et al.* (26), in a study of oral cancer in Shanghai, found an increased risk of oral cancer in individuals who consumed higher quantities of salted meat and fish, which was particularly evident in those who consumed few oranges and tangerines (OR, 11.73 for men and 7.23 for women). They also found an increased risk of laryngeal cancer with an intake of salted meat and fish (27). Winn *et al.* (28) reported an increase in the risk of oral and pharyngeal cancer in women with consumption of smoked fish (OR, 3.3; $P = 0.03$), smoked poultry (OR, 3.7; $P = 0.03$), and smoked beef (OR, 1.8; $P = 0.13$). Although they did not find an increased risk with nitrite-containing meats (corned beef, lunch meat, frankfurters, canned meat, and bacon), they did with pork products (ham or pork dried meats, bacon, sausage, brains, lunch meat, frankfurters, and canned meats). In a study from Brazil, however, Franco *et al.* (29) did not find an increased risk of oral cancer with frequent intake of smoked meats.

Concern has been raised in the past regarding the addition of nitrite to meat, poultry, and fish (4). Since nitrite may combine with nitrosable amines to form NDMA, foods with added nitrite tend to have elevated levels of NDMA. However, the linear correlation between nitrite and NDMA in this study was not particularly strong (Pearson product-moment correlation coefficient = 0.3). Although processed meats and fish tend to be high in both nitrite and NDMA, there are other foods which contain some nitrites but no measurable amounts of NDMA. These include beets, rhubarb, other vegetables, and most grains. The American Academy of Sciences estimated that one-third of the nitrites normally consumed are from cured meats and fish; one-third are from grains, cereals, and baked goods; and one-third are from other types of food (4). Endogenous formation of nitrite in saliva (from ingested nitrates) may contribute additionally to nitrite consumption, although we were unable to measure this. In this study, the odds ratios for nitrites differed from those of NDMA. In some instances the point estimates for esophageal cancer were stronger for high nitrite consumption than for high NDMA consumption (Tables 3 and 5). This may indicate that nitrites may combine with amines and amides to form other known animal carcinogens, such as *N*-nitrosodiethylamine or *N*-nitrosopyrrolidine, or this may simply reflect random variation in the point estimates.

Ascorbic acid is known to inhibit nitrosation (30). Individuals who consumed higher amounts of ascorbic acid were less likely to develop esophageal, laryngeal, and (possibly) oral cancers. Subjects with low ascorbic acid and high NDMA intake may be at particular risk of upper aerodigestive tract cancers. We were unable to ascertain whether the specific processed meats recorded in this study contained ascorbate. Some manufacturers add ascorbate to nitrite-containing meat products while others do not. It is unknown how this information would have affected the relative risk estimates since the proportions of cases and of controls who consumed ascorbate-containing meats were not known. Unfortunately, no information was collected regarding the methods of preparation (cooked *versus* raw) so that ascorbic acid degradation could be more adequately assessed.

Beer contained an appreciable amount of NDMA during the time frame of this study but has been reported to have nondetectable amounts of nitrite (31). Although beer manufacturers have been decreasing the amount of NDMA in beer since the early 1980s (32), the amount of NDMA in beer prior to the estimated onset of cancer in the study subjects was still quite high. Daily beer consumption was associated with an increased risk of upper aerodigestive tract cancer at each cancer site. Since beer contained both NDMA and ethanol (another known risk factor) we adjusted for total alcohol intake. After adjustment daily beer consumption yielded elevated odds ratios for each cancer site, although they were statistically significant only for esophageal and oral cancer (Table 1). Therefore, the elevated risk of upper aerodigestive tract cancer for NDMA consumption could not be attributed to a disproportionate number of subjects who consumed ethanol in the case group.

The results for esophageal cancer were somewhat different than those for laryngeal or oral cancer. Tea consumption affected the risk estimates for esophageal cancer. The odds ratios in relation to nitrate and nitrite consumption were lower in tea drinkers than in subjects who did not drink tea or drank it infrequently. In a recent review of tea drinking and cancer risk there appeared to be no correlation between esophageal cancer and tea consumed at normal temperatures (33). However, some studies have indicated a higher risk with high temperature tea (33). LaVecchia *et al.* (34) did not find a significant association between tea and esophageal cancer (relative risk, 1.0) or oral cancer (relative risk, 0.6), although the relationship between tea consumption and *N*-nitroso compounds was not reported. Notani and Jayant (35) found an increased risk of esophageal and pharyngeal cancer in India with frequent daily tea

consumption, although the temperature of the tea was not given.

Tea (*Camellia sinensis*) contains polyphenols, such as flavonoids, which have antioxidant properties (33). Hertog *et al.* (36) found in a European population that almost 50% of the dietary intake of flavonoids was from tea consumption. Both green and black teas have been found to inhibit nitrosation reactions in humans (37, 38). Han and Xu (39) reported that green and black tea inhibited esophageal tumors induced by *N*-nitrosomethylbenzylamine in rats. Other investigators have found similar results (40, 41). Yang and Wang (33) reported that 3–5 g of tea daily may block nitrosation in humans. In our study, intake of green and black tea was not recorded separately. Regular (nonherbal) tea was recorded and judging by its availability in grocery stores it is assumed that most of the subjects (95% of whom were Caucasian) drank black tea. Effect modification was not evident with the use of herbal teas, which are made from various herbs and not from *C. sinensis*.

Macrophages have been shown to produce nitrosamines *in vitro* (6); therefore, endogenous formation of *N*-nitroso compounds was considered. A history of oral infections was not related to increased cancer risk. In fact, a history of canker or cold sores was more prevalent in controls than in cases. A history of having canker sores, however, affected the relative risk estimates for esophageal cancer in relation to consumption of nitrate and nitrite (Table 5). Individuals who ever had canker sores were more likely to be at an increased risk of esophageal cancer if they consumed foods high in nitrites. Furthermore, the apparent protective effect for nitrate consumption was only evident in those who never had canker sores. It is not clear why a difference in odds ratios was seen for esophageal cancer and not for oral cancer, the site of the canker sores. Tricker and Preussman (2) indicated that nitrosamines generally produce systemic tumors distant from the site of application so that it may be possible for inflammation to stimulate reactions which affect other regions of the body. It is also possible that the presence of canker sores is a marker for other unknown factors not measured in this study. The etiology of *aphthous stomatitis* (canker sores) is unknown, although individuals with immunological disorders may be at higher risk (42).

The results of this study suggest a decreased risk of upper aerodigestive tract cancer with frequent consumption of nitrate-containing foods. It is reassuring to find that vegetables high in nitrates do not appear to adversely affect health, contrary to what might be expected from laboratory evidence. The results also suggest an increased risk of upper aerodigestive tract cancer with frequent consumption of foods containing NDMA. Furthermore, the associations between *N*-nitroso precursors and esophageal cancer may be modified by tea intake or by the presence of inflammation. In light of these findings and the laboratory evidence, additional studies in humans are warranted to determine if the consumption of processed fish and meats increase cancer risk and what factors may modify this risk. Such studies could be facilitated with wider availability of the concentrations of specific *N*-nitroso compounds in foods. Most food tables and databases from the United States do not contain amounts of *N*-nitroso compounds in foods routinely consumed by humans.

References

1. Bogovski, P., and Bogovski, S. Animal species in which *N*-nitroso compounds induce cancer. Int. J. Cancer, *27:* 471–474, 1981.

2. Tricker, A. R., and Preussmann, R. Carcinogenic *N*-nitrosamines in the diet: occurrence, formation, mechanisms and carcinogenic potential. Mutat. Res., *259:* 277–289, 1991.

3. Bartsch, H. *N*-nitroso compounds and human cancer: where do we stand? *In:* Relevance to Human Cancer of *N*-Nitroso Compounds, Tobacco Smoke and Mycotoxins, pp. 1–10. IARC Scientific Pub. No. 105. Lyon, France: International Agency for Research on Cancer, 1991.

4. Committee on Nitrite and Alternative Curing Agents in Food, National Academy of Sciences. The Health Effects of Nitrate, Nitrite, and *N*-Nitroso Compounds. Washington, DC: National Academy Press, 1981.

5. Tannenbaum, S. R. Endogenous formation of *N*-nitroso compounds: a current perspective. *In:* Relevance of *N*-Nitroso Compounds to Human Cancer: Exposures and Mechanisms, pp. 292–296. IARC Scientific Pub. No. 84. Lyon, France: International Agency for Research on Cancer, 1987.

6. Miwa, M., Stuehr, D. J., Marletta, M. A., Wishnok, J. S., and Tannenbaum, S. R. Nitrosation of amines by stimulated macrophages. Carcinogenesis (Lond.), *8:* 955–958, 1987.

7. Bartsch, H., Ohshima, H., and Pignatelli, B. Inhibitors of endogenous nitrosation: mechanisms and implications in human cancer prevention. Mutat. Res., *1202:* 307–324, 1988.

8. Lijinsky, W., and Taylor, H. W. Nitrosamines and their precursors in food. *In:* Origins of Human Cancer. Cold Spring Harbor Conferences on Cell Proliferation, Vol. 4., pp. 1579–1590. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1977.

9. Rogers, M. A. M., Thomas, D. B., Davis, S., Weiss, N. S., Vaughan, T. L., and Nevissi, A. E. A case-control study of oral cancer and pre-diagnostic concentrations of selenium and zinc in nail tissue. Int. J. Cancer, *48:* 182–188, 1991.

10. Waksburg, J. Sampling methods for random digit dialing. J. Am. Stat. Assoc., *73:* 40–46, 1978.

11. Panalaks, T., Iyengar, J. R., and Sen, N. P. Nitrate, nitrite, and dimethylnitrosamine in cured meat products. J. Assoc. Off. Anal. Chem., *56:* 621–625, 1973.

12. Panalaks, T., Iyengar, J. R., Donaldson, B. A., Miles, W. F., and Sen, N. P. Further survey of cured meat products for volatile *N*-nitrosoamines. J. Assoc. Off. Anal. Chem., *57:* 806–812, 1974.

13. Havery, D. C., Kline, D. A., Miletta, E. M., Joe, F. L., and Fazio, T. Survey of food products for volatile *N*-nitrosamines. J. Assoc. Off. Anal. Chem., *59:* 540–546, 1976.

14. Gough, T. A., Webb, K. S., and Coleman R. F. Estimate of the volatile nitrosamine content of UK food. Nature (Lond.), *272:* 161–163, 1978.

15. Breslow, N. E., and Day, N. E. The analysis of case-control studies. *In:* Statistical Methods in Cancer Research, Vol. 1. IARC Science Pub. No. 32. Lyon, France: International Agency for Research on Cancer, 1980.

16. Rogers, M. A. M., Thomas, D. B., Davis, S., Vaughan, T. L., and Nevissi, A. E. A case-control study of element levels and cancer of the upper aerodigestive tract. Cancer Epidemiol., Biomarkers & Prev., *2:* 305–312, 1993.

17. Ziegler, R. G. Vegetables, fruits, and carotenoids and the risk of cancer. Am. J. Clin. Nutr., *53:* 251S–259S, 1991.

18. Block, G., Patterson, B., and Subar A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr. Cancer, *18:* 1–29, 1992.

19. Bin, D. F., and Bresnick, E. Chemoprevention of nonnutrient components of vegetables and fruits. *In:* Cancer and Nutrition, pp. 221–260. New York: Plenum Publishing Corp., 1991.

20. Risch, H. A., Jain, M., Choi, N. W., Fodor, J. G., Pfeiffer, C. J., Howe, G. R., Harrison, L. W., Craib, K. J. P., and Miller, A. B. Dietary factors and the incidence of cancer of the stomach. Am. J. Epidemiol., *122:* 947–959, 1985.

21. González, C. A., Riboli, E., Badosa, J., Batiste, E., Cardona, T., Pita, S., Sanz, J. M., Torrent, M., and Agudo, A. Nutritional factors and gastric cancer in Spain. Am. J. Epidemiol., *139:* 466–473, 1994.

22. Boeing, H., Frentzel-Beyme, R., Berger, M., Berndt, V., Göres, W., Körner, M., Lohmeier, R., Menarcher, A., Männl, H. F. K., Meinhardt, M., Müller, R., Ostermeier, H., Paul, F., Schwemmle, K., Wagner, K. H., and Wahrendorf, J. Case-control study of stomach cancer in Germany. Int. J. Cancer, *47:* 858–864, 1991.

23. Buiatti, E., Palli,D., DeCarli, A., Amadori, D., Avellini, C., Bianchi, S., Bonaguri, C., Cipriani, F., Cocco, P., Giacosa, A., Marubini, E., Minacci, C., Puntoni, R., Russo, A., Vindigni, C., Fraumeni, J. F., and Blot, W. J. A case-control study of gastric cancer and diet in Italy. II. Association with nutrients. Int. J. Cancer, *45:* 896–901, 1990.

24. Chyou, P. H., Nomura, A. M. Y., Hankin, J. H., and Stemmermann, G. N. A case-control study of diet and stomach cancer. Cancer Res., *50:* 7501–7504, 1990.

25. Jain, M., Burch, J. D., Howe, G. R., Risch, H. A., and Miller, A. B. Dietary factors and risk of lung cancer: results from a case-control study, Toronto, 1981–1985. Int. J. Cancer, *45:* 287–293, 1990.

26. Zheng, W., Blot, W. J., Shu, X. O., Diamond, E. L., Gao, Y. T., Ji, B. T., and Fraumeni, J. F. Risk factors for oral and pharyngeal cancer in Shanghai, with emphasis on diet. Cancer Epidemiol., Biomarkers & Prev., *1:* 441–448, 1992.

27. Zheng, W., Blot, W. J., Shu, X. O., Gao, Y. T., Ji, B. T., Ziegler, R. G., and Fraumeni, J. F. Diet and other risk factors for laryngeal cancer in Shanghai, China. Am. J. Epidemiol., *136:* 178–191, 1992.

28. Winn, D. M., Ziegler, R. G., Pickle, L. W., Gridley, G., Blot, W. J., and Hoover, R. N. Diet in the etiology of oral and pharyngeal cancer among women from the southern United States. Cancer Res., *44:* 1216–1222, 1984.

29. Franco, E. L., Kowalski, L. P., Oliveira, B. V., Curado, M. P., Pereira, R. N., Silva, M. E., Fava, A. S., and Torloni, H. Risk factors for oral cancer in Brazil: a case-control study. Int. J. Cancer, *43:* 992–1000, 1989.

30. Tannenbaum, S. R., Wishnok, J. S., and Leaf, C. D. Inhibition of nitrosamine formation by ascorbic acid. Am. J. Clin. Nutr., *53:* 247S–250S, 1991.

31. Massey, R. C., Key, P. E., McWeeny, D. J., and Knowles, M. E. An investigation of apparent total *N*-nitroso compounds in beer. *In:* Relevance of *N*-Nitroso Compounds to Human Cancer: Exposures and Mechanisms, pp. 219–221. IARC Scientific Pub. No. 84. Lyon, France: International Agency for Research on Cancer, 1987.

32. Scanlan, R. A., and Barbour, J. F. *N*-nitrosodimethylamine content of US and Canadian beers. *In:* Relevance to Human Cancer of *N*-Nitroso Compounds, Tobacco Smoke and Mycotoxins, pp. 242–243. IARC Scientific Pub. No. 105. Lyon, France: International Agency for Research on Cancer, 1991.

33. Yang, C. S., and Wang, Z. Tea and cancer. J. Natl. Cancer Inst. *85:* 1038–1049, 1993.

34. LaVecchia, C., Negri, E., Franceschi, S., D'Avanzo, B., and Boyle, P. Tea consumption and cancer risk. Nutr. Cancer, *17:* 27–31, 1992.

35. Notani, P. N., and Jayant, K. Role of diet in upper aerodigestive tract cancers. Nutr. Cancer, *10:* 103–113, 1987.

36. Hertog, M. G. L., Hollman, P. C. H., Katan, M. B., and Kromhout, D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. Nutr. Cancer, *20:* 21–29, 1993.

37. Stitch, H. F. Teas and tea components as inhibitors of carcinogen formation in model systems and man. Prev. Med., *21:* 377–384, 1992.

38. Wang, H., and Wu, Y. The blocking effect of Chinese tea on *N*-nitrosation *in vitro* and *in vivo*. *In:* Nutrients and Cancer Prevention, pp. 546–549. Totowa, New Jersey: Humana Press, Inc., 1990.

39. Han, C., and Xu, Y. Chinese tea inhibits the occurrence of oesophageal tumours induced by *N*-nitrosomethylbenzylamine and blocks its formation in rats. *In:* Relevance to Human Cancer of *N*-Nitroso Compounds, Tobacco Smoke and Mycotoxins, pp. 541–545. IARC Scientific Pub. No. 105. Lyon, France: International Agency for Research on Cancer, 1991.

40. Xu, Y., and Han, C. The effect of Chinese tea on the occurrence of esophageal tumors induced by *N*-nitrosomethylbenzylamine formed *in vivo*. Biomed. Environ. Sci., *3:* 406–412, 1990.

41. Yoshizawa, S., Horiuchi, T., Fujiki, H., Yoshida, T., Okuda, T., and Sugimura, T. Antitumor promoting activity of (−)-epigallocatechin gallate, the main constituent of "tannin" in green tea. Phytother. Res., *1:* 44–47, 1987.

42. Gallin, J. I. Disorders of phagocytic cells. *In:* Harrison's Principles of Internal Medicine, pp. 278–283. New York: McGraw-Hill, Inc., 1987.